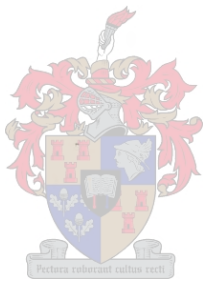
Susceptibility of five strains of vine mealybugs, *Planococcus ficus* (Signoret), to chlorpyrifos.

Owen De Wet



Assignment presented in partial fulfillment of the requirements for the degree of Master of Science in Agriculture at the University of Stellenbosch.

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Declaration

I, the undersigned, hereby declare that the work contained in this assignment is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

OPSOMMING

Kolonies van *Planococcus ficus* (Signoret), is versamel en geteel uit drie verskillende areas, Hexriviervallei, Robertson en Stellenbosch. 'n Bestaande insektarium kolonie van die Lanbou Navorsings Raad en 'n tafeldruif kolonie vanaf Nietvoorbij proefplaas is ook ingesluit in die studie. 'n Reeks konsentrasies van chlorpyrifos is topikaal aangewend aan individue van die verskillende kolonies. Die Stellenbosch populasie het die laagste LD_{50} getoon alhoewel dit nie betekenisvol verskil het van die LD_{50} van die insektarium – en Robertson kolonies nie. Die Hexriviervallei en tafeldruif kolonies se LD_{50} was betekenisvol hoër as die Robertson, Stellenbosch and insektarium kolonies. Alhoewel die relatiewe weerstand 1.5 was, sal dit waarskynlik nie tot 'n aansienlike beheermislukking in die veld lei nie. Nogtans dui dit op die potensiaal vir moontlike ontwikkeling van weerstand teen chlorpyrifos in die wingerdwitluis.

ABSTRACT

Colonies of *Planococcus ficus* (Signoret) were reared from three different areas, Hex River Valley, Robertson and Stellenbosch. An insectary colony and a table grape colony from Nietvoorbij experimental farm were also included in the study. A range of concentrations of chlorpyrifos was applied topically to individuals from the different colonies. The Stellenbosch population had the lowest LD_{50}, although it was not significantly different from that of the insectary and Robertson colonies. The Hex River Valley and table grape colonies had a significantly higher LD_{50} than the Robertson, Stellenbosch and insectary colonies, although the relative tolerance was 1.5, which would probably not result in significant control failure in the field. However, this does indicate that there is potential for the development of resistance to chlorpyrifos in the vine mealybug in South Africa.

ACKNOWLEGEMENTS

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CONTENTS

Susceptibility of five strains of vine mealybugs, *Planococcus ficus* (Signoret), to chlorpyrifos.

Introduction

The presence of vine mealybug, *Planococcus ficus* (Signoret), on bunches leads to table grape rejections and lowers wine quality. Mealybug development is directly related to temperature and favourable temperatures between 20 and 30 °C can lead to proliferation of vine mealybug populations during the growing season. Eggs are protected in an egg sack and at an average temperature of 25 °C, crawlers hatch within 7 to 10 days. Only the females feed on the vines and cause damage. The adult males do not have feeding mouthparts and have a lifespan of up to 3 days. At the beginning of the summer the crawlers move to new growth and later onto bunches (Walton 2001).

During recent seasons, there have been increasing reports of suspected chemical failure against *P. ficus,* following treatment with several organophosphates. No investigations have been made to determine the possibility of pesticide resistance by mealybug in South Africa. Vine mealybugs are found in cracks and under loose bark on the vines (Whitehead 1957). Therefore contact with the insecticide is not always assured (Grasswitz and Burts 1995). Furthermore, mealybugs produce honeydew and a soft waxy secretion which hinders direct contact with insecticides.

For efficient grape production in South Africa, chemical control is still essential. Sprays must be carefully timed to minimise the disruption of the most important natural enemy of *P. ficus*, *Coccidoxenoides peregrinus,* (Walton and Pringle 1999). Effective control of the vine mealybug would lead to a decrease in the occurrence of grapevine leafroll virus which is of great concern to the wine industry, and to a decrease in the number of infected table grape bunches.

Materials and Methods

Insects

Vine mealybugs were sampled from three vine production areas during the 2002/3 season in the Western Cape. These were Hex River Valley (33°30'E, 19°33'S, alt. 370 m), Robertson (33°49'E, 19°47'S, alt. 180 m) and Stellenbosch (33°54'E, 18°52'S, alt. 146 m) and a table grape vineyard (33°54'E, 18°52'S, alt. 146 m) on Nietvoorbij experimental farm. Mealybugs from a long-standing laboratory culture, established during the 1994 and 1995 season from various areas in the Western Cape, were also used. The mealybugs were reared on butternuts until sufficient numbers were available for testing.

Mealybugs from the same area were placed together in 60 x 50 x 37 cm wooden cages with three butternuts. Cages had a lid that clamped onto rubber strips (5 mm thick and 15 mm wide) to seal the container. For ventilation an 80 micron nytal gauze (0.2 m^2) was glued onto the lid. Cages were placed in a dark room maintained at a constant temperature of 24 °C and 75 % relative humidity. Butternuts were gradually added as the number of crawlers increased.

Chemical used

Organophosphates are the most commonly used insecticides for vine mealybug control. It was, therefore, decided that chlorpyrifos would be used as a representative insecticide in this study. This chemical is also widely used for mealybug control at present. A stock solution (1g/ml) of chlorpyrifos (technical-grade) was diluted to a range of concentrations (150; 225; 337.5; 507.25; 759.375 mg/ml) with acetone. This concentration range was determined in preliminary experiments.

Insecticide Bioassay

Adult female mealybugs were collected from butternuts using foreceps. Between 50 to 60 mealybugs were placed into glass petri dishes (14 cm in diameter) for each treatment.

The insecticide was applied ventrally to the mealybugs using a repeating microapplicator (Hamilton Company, Reno. Nevada). Treatments consisted of 0.5 µl/mealybug of one of the concentrations of chlorpyrifos. Acetone was used as a control. Following treatment, mealybugs were placed in a cooled incubator at 22.5 °C, relative humidity 60 % - 80 % and a 24 h photoperiod. Insect mortality was assessed 24 h after treatment. Mealybugs were considered dead if there was no movement of antennae or legs after 30 seconds of probing with a forceps.

Data Analysis

Probit analyses (Finney 1971), utilising POLO PC programme (LeOra Software 1987), were applied to the data to determine LD_{50}, LD_{90} and relative potency values of chlorpyrifos to the different populations. Slopes were arranged in increasing order for each data set and analysed to see if the lines were the same or parallel. If parallelism for a pair or group of lines was confirmed the next population's data were added until the assumption of parallelism was rejected. Relative potency was determined for groups of data with parallel lines (Finney, 1971). Correspondence analysis was performed on populations to identify possible groupings (Greenace, 1985). Data used in correspondence analysis were the percent mortality corrected for control mortality (Abbott 1925). The five concentrations were entered as column variables and the five colonies as row variables.

Results and Discussion

The probit lines for the Hex River Valley (Hex) and table grape (Tab) populations were the same (Figure 1, Table 1) and parallel to that of the Stellenbosch (Stel) population. However, the intercepts differed. The probit lines for the Robertson (Rob) and Insectary (Lab) populations were the same but not parallel to those of the other populations.

These flatter slopes of the Stel, Hex and Tab populations relative to those of the Rob and Lab populations (Table 1) indicated more genetic heterogeneity of the former populations in their reaction to chlorpyrifos (Finney 1971).

The Stel population was more sensitive than the other populations, having the lowest LD_{50} value (Table 1). However, the LD_{50} and LD_{90} fiducal limits overlapped with those of the Lab and Rob colonies indicating that all 3 colonies may be considered equally sensitive. The LD_{50} and LD_{90} of Hex and Tab populations were significantly higher than those of Lab and Rob populations indicating that the former populations were more resistant to chlorpyrifos than the latter. The Hex and Tab colonies were 1.5 times more resistant than the Stel population (Relative potency = 1.5; 95 % fiducal limits = 1.199 to 1.940). The LD_{50} values of Rob and Lab were 1.2 times higher than that of Stel population, but because the lines were not parallel, the fiducal limits for relative potency could not be estimated. However, the fiducal limits for both the LD_{50} and LD_{90} of the Stel and the Rob & Lab populations overlapped (Table 1). Therefore, it can be assumed that the Stel and Rob and Lab populations were equally sensitive to chlorpyrifos.

In the correspondence analysis 81% of the inertia was described in the first dimension (X-axis). The colonies to the left of the centroid (Stel, Lab, Hex) had higher mortalities at the two lowest concentrations than the two colonies to the right of the centroid (Rob, Tab). Therefore, the first dimension appeared to describe the reaction of the strains to the low doses of chlorpyrifos. The second dimension accounted for 13 % of the inertia. The Hex and Tab strains were below the

centroid, while the other three strains were above the centriod. The Hex and Tab strains had the highest LD_{50} and LD_{90} values (Table 1). They were, therefore, the most tolerant to chlorpyrifos.

Table 1. Probit regression equations, LD_{50} and LD_{90} values (mg/ml) with their 95% fiducal limits for the Stellenbosch (Stel), Robertson and Insectary (Rob & Lab) and Hex River Valley and Table grape (Hex & Tab) populations.

Locality	Probit regression	LD_{50}	95% Fiducal Limits	LD_{90}	95% Fiducal Limits
Stel	-4.8721+ 3.9130 (x)	220.0	177.8 - 267.0	467.7	379.7 - 609.7
Hex & Tab	-4.1654 + 3.9130 (x)	333.4	298.7 - 379.8	708.6	596.5 - 902.6
Rob & Lab	-8.2085 + 5.4499 (x)	265.2	249.5 - 281.6	455.8	418.6 - 507.0

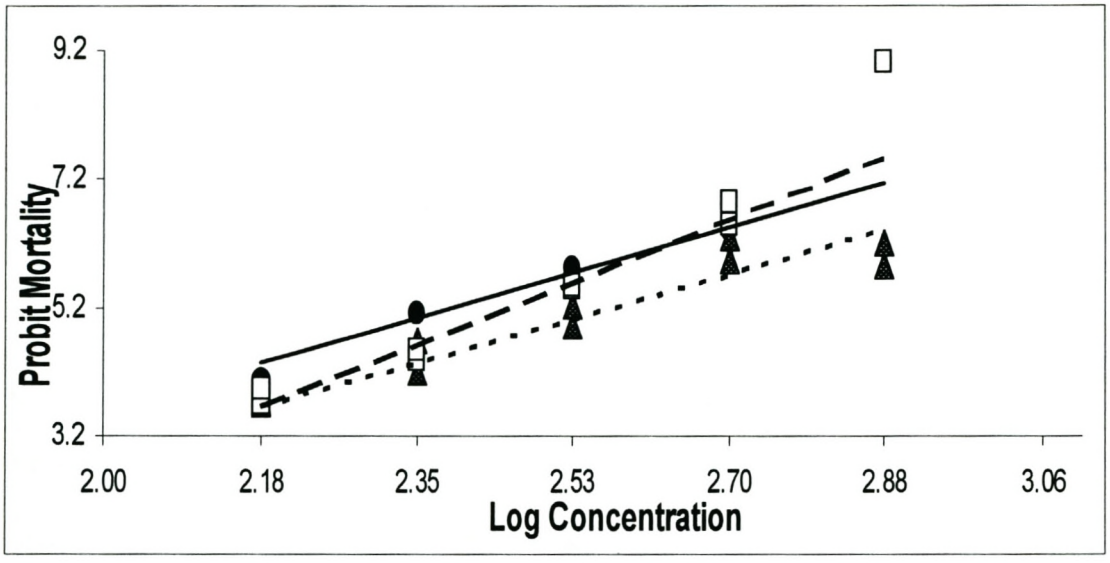

Figure 1. Probit regression lines for the Stellenbosch (• ——), Robertson and Insectary (□ – – –) and Hex River Valley and Table grape (▲ - - - -) populations.

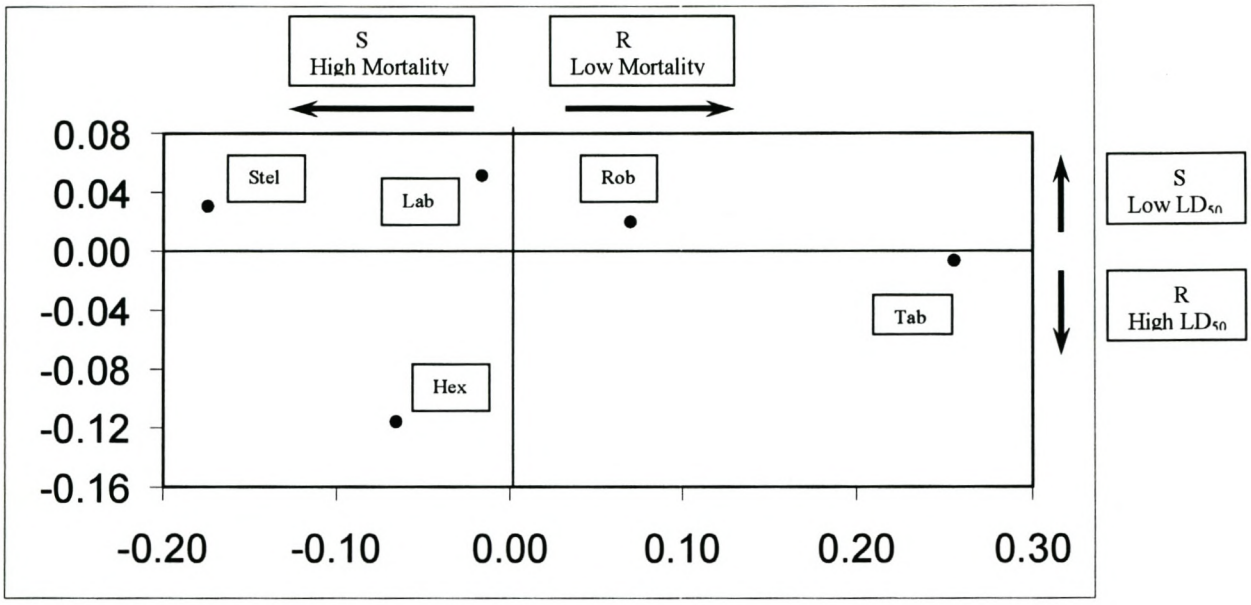

Figure 2: The first two principal axes of a correspondence analysis of the reaction of the Stellenbosch (Stel), Hex River Valley (Hex), Insectary (Lab), Robertson (Rob) and Table grape (Tab) populations to five concentrations of chlorpyrifos. S = susceptible and R = resistant.

Conclusion

The registered field dose of chlorpyrifos (EC 480g/l AI at 200ml/500l/Ha in winter) is lower than the doses used in this study. However in these experiments mortality was determined after 24 hours, while in the field exposure is considerably longer. Therefore results from the laboratory study should not be compared with these from the field. Surprisingly the long-standing insectary colony was not the most susceptible colony. Although the level of resistance detected was low, it does indicate that resistance to chlorpyrifos is possible.

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